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(54) Title: CHOLESTEROL REDUCING STANOL COMPOSITIONS, PREPARATION AND METHOD OF USE

(57) Abstract: A composition comprising a mixture of a phytostanol and/or phytostanol ester and a surfactant(s). The surfactants are selected from the group consisting of anionic, cationic, nonionic, and zwitterionic surfactants. The phytostanol is selected from the group consisting of sitostanol, campestanol, 22,23 dihydrobrassicastanol, and mixtures thereof. The phytostanol esters are derivatives of the aforementioned phytostanols. The invention is also directed to a method of making the disclosed compositions, and to non-fat containing food products including the disclosed compositions.

Cholesterol Reducing Stanol Compositions, Preparation and Method of Use

BACKGROUND

Field of the Invention

This invention relates to plant stanol compositions and its derivatives for reduction of cholesterol absorption. More particularly, the invention provides compositions containing a phytostanol and/or a phytostanol ester and surfactant(s) that are useful for reducing cholesterol absorption. The invention also relates to methods of preparing such compositions for reducing cholesterol absorption.

Related Background Art

Cholesterol, while an essential nutrient for humans, is well known to be a leading cause of death in the United States and most countries around the world. Many foods consumed today have high cholesterol content. Once the cholesterol reaches the small intestine it can be absorbed which results in an increase in serum cholesterol levels. The serum cholesterol is well-known to be deposited in various parts of the circulatory system, for example, in soft tissues. The long-term accumulation or build-up of cholesterol deposits leads to atherosclerotic disease.

By reducing cholesterol content of food, as well as inhibiting the absorption of cholesterol, it has been possible to reduce serum levels of cholesterol. One of the areas that has been explored to control serum cholesterol levels is the use of dietary supplements, such as cholestyramine resin, probucol, colestipol HCl, nicotinic acid, mevinolin, pectin, guar gum, and oat bran. Another area, that has received considerable attention, has been the development of food additives that reduce the absorption of cholesterol in the small intestine. Prevention of the absorption of cholesterol results in lower levels of cholesterol in the blood and thus helps to prevent the formation of atherosclerotic plaques.

Plant sterols have been found to be particularly effective at reducing serum cholesterol levels. In particular, studies conducted employing beta-sitosterol were found to produce significant reductions (17%) in the amounts of cholesterol in the blood (Farquhar, J.W. et al., Circulation, 14, 77-82 (1956)). However, large doses of beta-sitosterol were required, 12-18 grams per day. This is a major impediment to the use of beta-sitosterol for cholesterol reduction. A related class of compounds, plant stanols (saturated plant sterols), have also been found to be effective for reducing cholesterol absorption. It has been postulated that plant stanols block cholesterol absorption by competing with cholesterol for bile acid micellerization. As a result of this competition between plant stanols and cholesterol, it is believed that plant stanols displace cholesterol from the micellar phase and

thereby prevent its absorption in the small intestine.

Plant stanols and sterols have represented particularly attractive classes of compounds for use in lowering serum cholesterol levels, since they are natural components of vegetable fats and oils. Additionally, plant stanols and sterols are absorbed in very small quantities compared to the absorption of bile and dietary cholesterol. In fact, sitostanol is considered to be practically unabsorable, i.e., less than 5%. Although, plant sterols and stanols are attractive as cholesterol absorption inhibitors, they have proven to be difficult to formulate. A major factor in these difficulties has been the fact that plant stanols are virtually insoluble in water. Considerable efforts have been made to develop plant sterol or stanol preparations that can easily be formulated with consumer food products.

U.S. Patent No. 5,244,887 is directed to plant stanol food additives for reducing the absorption of cholesterol in the gastro-intestinal tract. The greatest effectiveness was obtained when the stanols were evenly distributed in finely divided form throughout the food product or beverage to which it is added. This was accomplished by dissolution of the stanols or by suspension of the stanols in an emulsion. Solubilizing agents listed for the stanols include vegetable oil, monoglycerides, diglycerides, triglycerides, tocopherols, and the like and mixtures thereof. Suspensions or emulsions of stanols include water, alchohols, polyols, and other edible compounds. Dispersing agents may be used to aid in the formation of suspensions, such as lecithin,

polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, polysorbate 85, sodium lauryl sulfate, and the like. The stanol food additives are used with cholesterol containing foods, such as meats, eggs and dairy products.

EP 0 897 671 discloses aqueous dispersions of high melting lipids, such as plant sterols, with non-sterol emulsifiers. Emulsifiers used to disperse the high melting lipids include polyglycerol esters and tweens, especially polysorbate 60. Mono- and diglycerides are also mentioned as suitable emulsifiers. The dispersions have reduced size on the order of 15 microns or lower. The dispersions are said to be useful in spreads and other food products. Additionally, the dispersions provide structure to the food products and their use can apparently permit minimization or elimination of saturated fats and trans fatty acids.

There is a continuing need for formulations which lower serum cholesterol levels by preventing or significantly reducing the absorption of cholesterol. Such formulations would desirably be able to be delivered in a variety of ways to individuals, e.g., as an additive to food products or as a pill for oral administration. Furthermore, in order for the formulations to be most effective in lowering cholesterol absorption, the formulation must reach the gastrointestinal tract so that they can be rapidly and efficiently solublized in the micellar phase thereby preventing the absorption of cholesterol. It has now been discovered that formulations comprising a plant stanol or a plant stanol ester and a surfactant(s) are effective at

inhibiting the absorption of cholesterol.

SUMMARY OF THE INVENTION

Compositions for inhibiting the absorption of cholesterol are disclosed. The compositions comprise a plant stanol and/or a plant stanol ester and a surfactant(s). The surfactant(s) is selected from the group consisting of anionic, cationic, nonionic and zwitterionic surfactants. Examples of plant stanols that may be employed include sitostanol, campestanol, 22, 23 dihydorbrassicastanol, and clionastanol. The compositions of the present invention provide an effective method of reducing cholesterol without any adverse side-effects.

The compositions of the present invention can be used as food additive compositions for reducing cholesterol absorption from foods. The food additive composition may be employed in small quantities making it convenient for use and also an inexpensive method to reduce cholesterol absorption. The food additive compositions of the present invention are storage-stable for extended periods of time. The food additive compositions may be added before, during or after cooking with foods. The food additive compositions may be added to food products during production prior to sale to the consumer. Advantageously, the compositions of the present invention can be used in non-fat containing foods.

The present invention also contemplates various combinations

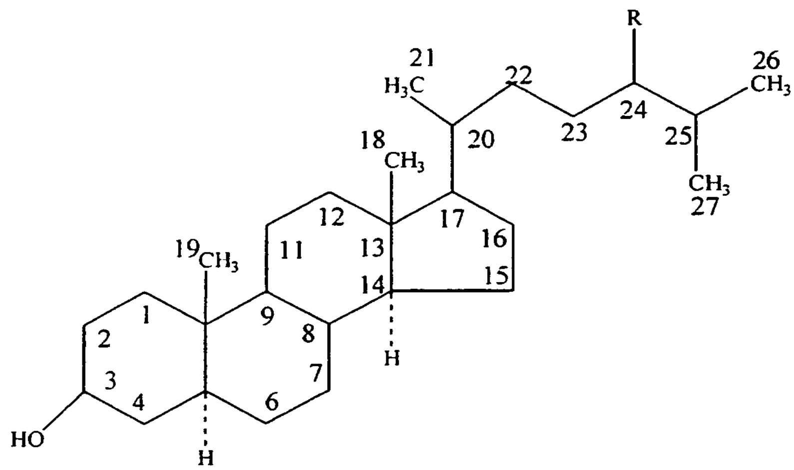
of the foregoing surfactant(s) and a phytostanol and/or a phytostanol ester being administered orally in any of the usual solid forms such as pills, tablets, capsules or powders, including sustained release preparations.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the use of plant stanol and/or plant stanol ester compositions that inhibit the absorption of cholesterol. More particularly, the invention relates to the formation of water soluble/dispersible stanol and/or stanol°ester systems comprising a plant stanol and/or a plant stanol ester and anionic, cationic, nonionic, or zwitterionic surfactant systems, which reduce cholesterol absorption.

The terms "phytostanols" and "stanols" are used herein interchangeably. The term "stanols" as used herein refers to plant sterol derivatives in which all carbon-carbon bonds in the rings are saturated. The principal stanols of the present invention are those which are composed of 28 or 29 carbon atoms. Four major plant stanols are beta-sitostanol, campestanol, 22,23 dihydrobrassicastanol, and clionastanol. The Lipids, Vol. 1, Deuel, H. J., Jr., Interscience Publishers, 1951, N.Y., pp. 348-361.

These four stanols have the following structure



where R is CH_3 for campestanol and its epimer, 22,23 dihydrobrassicastanol and where R is C_2H_5 for sitostanol and its epimer, clionastanol. The C_{28} stanols campestanol and 22, 23 dihydrobrassicastanol differ only by their steric configuration at C_{24}. The C_{29} stanols, likewise, differ only by the steric configuration at C_{24}. Alternate nomenclature for clionastanol is (3 beta, 5 alpha, 24S)-Stigmast-5an-3-ol; sitostanol is (3 beta, 5 alpha, 24R)-Stigmast-5an-3-ol; campestanol is (3 beta, 5 alpha, 24R)-Ergost-5an-3-ol; 22, 23 dihydrobrassicastanol is (3 beta, 5 alpha, 24S)-Ergost-5an-3-ol. It will also be appreciated that modifications of the plant stanols are also well within the scope of the present invention, for example, small side chains.

The terms "phytostanol ester" and "stanol esters" are used

herein interchangeably. The term "stanol ester" as used herein refers to plant stanols that has been modified to form a plant stanol ester derivative. These derivatives are well known in the art and are described in U.S. Pat. Nos. 4,588,717, 5,270,041, and 5,958,913, International applications WO 98/06405, and WO 99/25362, European Application EP 911385, and H. Gylling et al., Journal of Lipid Research, 40, 593-600 (1999), the disclosures which are hereby incorporated by reference.

Stanols are found in small quantities in nature in many plants, e.g., wheat, rye, and corn. As a result, this is not a particularly good source of large quantities of stanols due to the large cost associated with extraction of sufficient quantities of stanols. A more cost effective method to obtain large quantities of stanols is by hydrogenation of the much more abundant plant sterols. Many hydrogenation methods for plant sterols are well-known by those of ordinary skill in the art. For example, plant sterols can be converted into stanols by hydrogenation techniques that employ Pd/C catalyst in organic solvents (Augustine, R.L. et al., Org. Prep. and Proc. 1: 107-109, (1969)).

A larger number of inexpensive sources of plant sterols are known. These include, vegetable oils, vegetable oil sludge, vegetable oil distillates, and other plant oil sources such as tall oils. For example, a preparation of sterols from vegetable oil sludge by using solvents such as methanol is taught in U.S. Pat. No. 4,420,427. Sterols isolated from

plant sources are usually mixtures of several different sterols, hydrogenation leads to a mixture the corresponding stanols. Sterols, which differ only by the degree of unsaturation in the carbon bonds of the ring or side chains, upon hydrogenation usually produce stanols which differ only in epimeric centers such as the C_{24} carbon.

One preferred plant stanol, sitostanol, may be obtained by hydrogenation of sitosterol. Sitosterol may be obtained from cold pressed wheat germ oil, soy extract, or rice extract. (It will be appreciated that natural sitosterol contains about 40% alpha-sitosterol and about 60% beta-sitosterol. Both the alpha and beta forms of sitosterol may be used to form sitostanol for use in the present invention.) Particularly preferred stanol comprises a minimum of 63 weight percent sitostanol, a maximum of 35 weight percent campestanol and a minimum of 93 weight percent of sitostanol and campestanol.

It should also be noted that hydrogenation of plant sterols may leave some amounts of unreacted sterols. Although, the hydrogenation results in a majority of stanol production. For the purposes of the present invention, it is acceptable to have small residual amounts of unreacted sterols, generally less than about 10%. It will be readily apparent to those of ordinary skill in the art that amount of residual sterol present may be substantially reduced (<1.5%) depending on the solvents and reaction conditions employed. However, if it is desired to have a stanol free of unreacted sterols, then a pure sterol preparation may be employed.

The surfactants to be employed according to the invention can be anionic, cationic, nonionic, zwitterionic and mixtures thereof. Suitable anionic surfactants include AOT, also known as sodium dioctylsulfosuccinate or sodium docusate, ammoniated glycyrrhizin, and sodium stearoyl lactylate. Nonionic surfactants include, polyoxyethylene castor oil (cremophor EL polyoxyl 35 castor oil), polyethylene glycol "PEG" (low molecular weight 1000 to 4000), diacetyl lactic acid of esters of mono- and diglycerides, diacetyl tartaric acid esters of mono- and diglycerides, monosodium phosphate derivatives of mono- and diglycerides, ethoxylated mono- and diglycerides, polyethylene glycol (8) stearate, polyethylene glycol (40) stearate, sorbitan esters of fatty acids, quillaja saponin, ethylene oxide propylene oxide block copolymers, vitamin E TPGS (d-alpha tocopheryl poyethylene glycol 1000 succinate), fatty alcohols and sucrose fatty acid esters, such as sucrose stearate, sucrose distearate, sucrose palmitate.

Preferred fatty alcohols include but are not limited to the following, 1-decanol ($CH_3(CH_2)_8CH_2OH$) also known as n-decyl alcohol, 1-dodecanol ($CH_3(CH_2)_{10}CH_2OH$) also known as dodecyl or lauryl alcohol, 1-tetradecanol ($CH_3(CH_2)_{12}CH_2OH$) also known as myristyl alcohol, 1-hexadecanol ($CH_3(CH_2)_{14}CH_2OH$) also known as cetyl or palmityl alcohol, 1-octadecanol ($CH_3(CH_2)_{16}CH_2OH$) also known as stearyl alcohol, 9-octadecen-1-ol ($CH_3(CH_2)_7CH{=}CH\ (CH_2)_7CH_2OH$) also known as oleyl alcohol, 1-eicosanol ($CH_3(CH_2)_{18}CH_2OH$) also known as arachidyl alcohol, 1-docosanol ($CH_3(CH_2)_{20}CH_2OH$) also known as behenyl alcohol, 1-hexacosanol ($CH_3(CH_2)_{24}CH_2OH$), 1-octacosanol ($CH_3(CH_2)_{26}CH_2OH$)

also know as octacosyl alcohol (wheat leaf wax), 1-triacontanol ($CH_3(CH_2)_{28}CH_2OH$) also known as melissyl alcohol (beeswax as stearate). A particularly preferred fatty alcohol is 1-octadecanol.

Zwitterionic surfactants include, hydroxylated lecithin and the like.

Other anionic surfactants such as sodium stearate, sodium palmitate, sodium laurate, sodium myristate, sodium linoleate, and potassium oleate may also be employed. Additional nonionic surfactants that may be included in the compositions of the present invention include polyglycerol esters and tweens, polysorbate 20 (tween 20), polysorbate 40 (tween 40), polysorbate 60 (tween 60), polysorbate 80 (tween 80), polysorbate 85 (tween 85), fatty acids such as oleic acid ($C_{17}H_{33}COOH$), stearic acid ($C_{17}H_{36}COOH$), and palmitic acid ($C_{15}H_{31}COOH$), triglycerides $CH_3(CH_2)_6COOH$ and $CH_3(CH_2)_8COOH$ and mixtures thereof (e.g., MCT oil).

Naturally occurring polymers such as guar gum, karaya gum, gum arabic, carrageenan, xanthan gum, dextran, maltodextrin, chondroitin sulfate, polyglycerol esters of fatty acids commercially known as Polyaldo, succinoglucan and hyaluronic acid may also be employed in the compositions of the present invention. Synthetic polymers such as poly(vinyl alcohol), poly(vinyl pyrrolidone), hydroxypropyl methyl cellulose, and sodium carboxymethyl cellulose may also be employed in the compositions of the present invention.

Other fatty acids, that may be employed in the present invention, include caprylic, capric, lauric, myristic, myristoleic, palmitoleic, oleic, ricinoleic, linoleic, linolenic, eleostearic, arachidic, arachidonic, behenic and erucic acid. The fatty acids of the present invention can be derived from naturally occurring or synthetic fatty acids; they can be saturated or unsaturated, including positional and geometric isomers, depending on the desired physical properties, for example liquid or solid.

In one preferred embodiment the composition of the present invention comprises a mixture of a phytostanol and/or a phytostanol ester and a surfactant selected from the group consisting of sodium docusate, ammoniated glycyrrhizin, polyoxyethylene castor oil, polyethylene glycol, diacetyl lactic acid esters of mono- and diglycerides, diacetyl tartaric acid esters of mono- and diglycerides, monosodium phosphate derivatives of mono- and diglycerides, ethoxylated mono- and diglycerides, quillaja saponin, ethylene oxide propylene oxide block copolymers, vitamin E TPGS, hydroxylated lecithin and mixtures thereof. The composition of the present invention may further comprise a surfactant selected from the group consisting of sodium salts of fatty acids, fatty alcohols, polyethylene glycol (8) stearate, polyethylene glycol (40) stearate, sucrose fatty acid esters, tween and mixtures thereof.

One preferred embodiment of the present invention comprises a mixture of a phytostanol and/or a phytostanol ester and fatty acid alcohol, preferably, octadecanol. Another

preferred embodiment of the present invention comprises a mixture a phytostanol and/or a phytostanol ester, Tween 60 and PEG. Other preferred embodiments include a phytostanol and/or a phytostanol ester, fatty acid alcohol and sucrose fatty acid ester, such as Crodesta.

One preferred embodiment of the present invention provides a method of reducing cholesterol absorption in humans which comprises orally administering an effective amount of a composition of the present invention. The invention also provides for a method for reducing serum cholesterol levels comprising administering a mixture of a phytostanol and/or a phytostanol ester and a surfactant(s).

In another embodiment of the present invention, emulsifiers may be used in the formulation of dispersible stanol and/or stanol ester systems. Preferred emulsifiers include a variety of phospholipids, phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), N-acylphosphatidyl ethanolamine (NAPE), phosphatidyl serine (PS), phosphatidyl inositol (PI), phosphatidyl glycerol (PG), diphosphatidyl glycerol (DPG), phosphatidic acid (PA) and plasmalogen. These and other phospholipids are described, for example, in Szuhaj and List (eds.), Lecithins, American Oil Chemists Society (1985) ("Szuhaj and List"), incorporated herein in its entirety by reference.

The phospholipids may be used individually or in various combinations, and may be obtained from "natural" sources (e.g., soybean lecithin) or from chemical synthesis. The

phospholipids may be in the form of relatively unpurified mixtures of phospholipids and other constituents (e.g., crude commercial lecithins obtained from the refining of soybean oil and other vegetable oils such as sunflower and canola), or may be purified to various degrees. In addition, phospholipids including those found in crude soybean lecithins or other crude commercial lecithins may be chemically modified. Lecithins, other phospholipid preparations, or individual phospholipids purified from natural sources or obtained by chemical synthesis, contain one or more functional groups susceptible to chemical modification, e.g., carbon-carbon double bonds, esters, phosphonate esters, amines and hydroxyl groups. Chemical modification of phospholipids can be compatible with the present methods. Thus, phospholipids that have been acetylated, hydroxylated, hydrolyzed (e.g., to produce lysophospholipids), hydrogenated, halogenated, phosphorylated, sulfated epoxidated, ethoxylated, or otherwise modified are potentially useful in the present methods and are included within the meaning of the term "phospholipid" as used herein. Various natural and synthetic phospholipids, including various types of lecithins, may be obtained commercially, for example Ultralec from ADM Corp., and other lecithins may be obtained from CALBIOCHEM®, La Jolla, Calif., USA and from SIGMA® Chemical Company, St. Louis, Mo., USA.

In common usage, the term "lecithin" refers to the entire phospholipid fraction obtained from natural sources such as soybean, cotton seed, corn, wheat germ, oat, barley,

sunflower, rapeseed, canola, linseed, peanut, palm kernel, egg yolk, milk and brain. Generally these fractions include a mixture of polar and neutral lipids with a polar lipid content (as defined by insolubility in acetone) of at least 50%. The art has also used the term "lecithin" as the common name for phosphatidyl choline. The term "lecithin"as used herein refers to the first usage, i.e., the entire phospholipid fraction obtained from selected vegetable oils or other appropriate sources. See, Chapter 2 of Szuhaj and List. It is to be noted, however, that phosphatidyl choline is an appropriate phospholipid for use in the present methods, either alone or in combination with other phospholipids.

Commercial soybean lecithin, a preferred source of phospholipids, is obtained from the refining of soybean oil. Crude soybean oil generally contains about 1.0 to 3.0 weight percent phospholipids. When the crude oil is refined, the first step generally is to remove the phospholipids. This step, often called "degumming," is accomplished by first adding water to the crude oil. The water hydrates the phospholipids and makes them less soluble in the oil. The denser phospholipids and water are then separated from the less-dense oil, typically by centrifugation. Removal of the water from the dense phase results in a product having approximately equal amounts of phosphatidyl choline, phosphatidyl ethanolamine, and inositol phosphatides. Partially refined soybean oil is commonly added back to produce a liquid product that is flowable at room temperature (sometimes called "fluidized lecithin").

Commercial fluid soybean lecithin contains about 50 to about 65 weight percent phospholipids and a small amount (generally less than about 5 weight percent) of various carbohydrates, mineral salts, protein materials, free fatty acids, sterols, and water. The remainder of commercial soybean lecithin is soybean oil.

Various lecithin powders enriched for phospholipid content are available commercially and may also be used in the present methods. Such lecithin powders are also within the scope of the term "lecithin" as used herein. The powders are typically derived by fractionation, for example acetone fractionation, of crude lecithins such as commercial soybean lecithin, and may contain from about 60% to over 95% phospholipid.

Another commercial source of phospholipids is the class of products, resulting from modification of soybean lecithin to improve its hydrophilic properties. Various approaches have been taken to effect these modifications. For example, soybean lecithin may be chemically or ezymatically modified, e.g., via reaction with maleic anhydride. Certain components may be removed from commercial soybean lecithin. Alternatively, another approach is to add various components, for example nonionic emulsifiers, to the commercial soybean lecithin. Such emulsifiers include, without limitation, polyoxyalkylene monoglyceride, polyoxyalkylene diglycerides, and the polyoxyethylene derivatives of partial fatty acid esters. These modified lecithins are also included in the term "lecithin" as used

herein.

Hydroxylated lecithin is a preferred embodiment of the phospholipids used in the invention. Hydroxylated lecithin is prepared by hydroxylating the double bonds in the fatty acids attached to the phospholipids and glycolipids of lecithin, which can be carried out by reaction with hydrogen peroxide and a weak acid such as lactic acid. Although not wishing to be bound by theory, it is believed that hydroxylation is not specific and can occur at any double bond within any of the lipids. The degree of hydroxylation is typically about 10% but can be varied by methods known to those of ordinary skill in the art.

The compositions of the present invention are preferably formed into a fine dispersion using melt processing. One particular preferred method of melt processing comprises dry-mixing a phytostanol and/or a phytostanol ester and a surfactant(s) together with a stirring device such as a mechanical stirrer, shear mixer, vibrational mixer or sonicator. The mixture is then heated to a temperature sufficient to melt same, but not so high as to degrade the phytostanol, phytostanol ester or surfactant(s). The resulting mixture is then rapidly cooled, e.g., liquid nitrogen, to form a salt like material. While not wishing to be limited by theory, it is believed that the step of melt blending the phytostanol and/or phytostanol ester and surfactant(s) prior to rapid cooling facilitates the formation of a composition that is in a finely dispersed state that is able to reach the small intestine thereby

being able to inhibit the absorption of cholesterol.

An alternative variation on the melt processing described above comprises the addition of a surfactant(s) to a phytostanol and/or phytostanol ester melt. This may be desirable in cases where the surfactant(s) is thermally unstable and would not survive prolonged heating at high temperatures, i.e., thus the residence time for the surfactant(s) is reduced by its later addition to phytostanol melt. Once the surfactant(s) has been added to the melt, the mixture can be treated in a fashion similar to that described previously.

A further alternative procedure to melt blending for forming the compositions of the present invention is high pressure melting. This procedure is also desirable for blending heat sensitive surfactant(s) and phytostanol and/or phytostanol esters. It has been discovered that by using a mixing or compression means allowing for increasing the pressure on the ingredients, the surfactant(s) phytostanol and/or phytostanol esters will melt blend at ambient temperatures. Homogenous mixtures of heat sensitive surfactant(s) and phytostanol and/or phytostanol esters can therefore be formed while avoiding temperatures at which thermal decomposition of ingredients will occur.

One embodiment of high pressure melting is roller compaction, where the surfactant(s) and phytostanol and/or phytostanol ester are mixed together as described previously. The mixture is then compressed together using a

roller such that the pressure exerted on the mixture is high enough to result in the flow of the ingredients and surface sintering of the mixture results.

An additional embodiment of high pressure melting is extrusion. For example, in an extrusion process, a loosely packed powder mixture of surfactant(s) and phytostanol and/or phytostanol ester, is propelled continuously along a screw through regions of high pressure and controlled temperature. Shear forces from the screw melt and mix the material into a continuous stream of molten material, which is then forced through a die.

The mixture resulting from the high pressure melting process is typically a soft, pliable solid material that can be further processed by cooling to solidify followed by breaking into chips or milling to form a uniform powder for use in the formation of products as described herein.

Mixtures of a phytostanol and/or a phytostanol ester and surfactant(s) may also be processed using solution processing or steric stabilization. Use of solution processing is particularly effective for use with surfactant(s) that are thermally stable. The phytostanol and/or phytostanol ester and surfactant(s) are generally dry mixed together, although this is not always necessary, the resulting solid mixture is then dissolved in an organic solvent, such as methylene chloride. The solvent is then removed to provide an amorphous/dispersible solid form.

Steric stabilization provides an effective method to form a dispersible solid form of a phytostanol and/or phytostanol ester and surfactant(s). A fine dispersion of a phytostanol and/or phytostanol ester is added to water containing the surfactant(s). The surfactant(s) in the water help to keep the stanol(s) in suspension. Once a finely divided suspension has formed the water is evaporated off leaving a more readily dispersible solid form.

Preferred embodiments of the present invention include a mixture of from about 10 to about 99.99 weight percent of a phytostanol and/or phytostanol ester and from about 0.01 to about 90 weight percent of a surfactant(s), preferably from about 40 to about 95 weight percent of a phytostanol and/or phytostanol ester and from about 5 to about 60 weight percent of a surfactant(s), and more preferably about 95 weight percent of a phytostanol and/or phytostanol ester and about 5 percent by weight percent of a surfactant(s).

In one preferred embodiment of the present invention, the compositions of the present invention comprise a phytostanol and/or phytostanol ester and at least two surfactants. Such a mixture comprises from about 10 to about 99.99 weight percent of a phytostanol and/or phytostanol ester and the sum of the at least two surfactants is from about 0.01 to about 90 weight percent, preferably such a mixture comprises from about 40 to about 95 weight percent of a phytostanol and/or phytostanol ester and the sum of at least two surfactants is from about 5 to about 60 weight percent, and more preferably about 95 weight percent of a phytostanol

and/or phytostanol ester and the sum of at least two surfactants is about 5 weight percent.

Desirable characteristics of food additive compositions for reducing cholesterol absorption include absence of side effects, efficacy without absorption of the compound, stability at cooking temperatures, stability in storage and in oxidizing environments, low cost, availability, and small dose requirements.

The compositions of the present invention may be orally administered in a variety of forms: uncoated tablets, coated tablets such as film, sugar or gelatin coated, chewable tablets, swallowable tablets (capsules), effervescent tablets, immediate release tablets, sustained (controlled or modified) release tablets; soft gelatin capsules either liquid (non-aqueous) or paste (slurry); hard gelatin capsules in powder (granulation), bead, tablet, liquid, semi-solid, sprinkle (immediate or controlled release) forms; oral liquids such as aqueous, emulsions or suspension; sachets (packages) in powder, granule or sprinkle (immediate or controlled release) forms. Other forms for administering the compositions of the present invention include syrup, fruit beverages, or fruit gelatines.

Although the composition of the invention may be used in various embodiments it may be said, one preferred embodiment is when the compositions of the present invention are evenly distributed in finely divided form throughout the food

product or beverage to which it is added. The compositions of the present invention may be added to food products or beverages prior to purchase by the consumer for consumption. Alternatively, the compositions of the present invention may be purchased in bulk form or individually wrapped packages, e.g., 8 oz. servings. A serving from the bulk form, e.g., 8 oz., or the contents of a package may be added to a glass of cold or hot water or other beverage, stirred to dissolve the contents, prior to consumption. Typical beverages include the following, instant ice tea in Orange Pekoe, English Breakfast, Passion Fruit and Hisbiscus Flavors, powdered soft drinks such as Crystal Light and Contry Time Lemonade, Instant Iced Coffee in flavors such as Mocchacchino, Hazelnut, French Vanilla, and Hot Chocolate and Fruit Smoothies.

The compositions of the present invention may also be included in mini-sweets. The mini-sweets will typically be consumed after any and all meals of each day. The mini-sweets will typically contain between 25 and 60 calories and 1 to 3 grams of fat. Mini-sweets include the following varieties: chocolate chews; caramel chews; hard candies such as cinnamon, butterscotch, coffee and fruit flavors; chocolate truffles such as hard dark chocolate on the outside filled with hazelnut creme, irish creme or cappucinno creme; brownie bites; cookie chews in peanut butter, chocolate chip or ginger snaps; granola/nutrition bar miniatures such as chocolate covered oat and peanut butter; chewy breath mints; mint meltaways; and mini-pudding. Mini-sweets may be prepared in individually

wrapped packages or in larger containers for multiple uses.

The method by which the novel food additive composition, i.e., the stanol and/or stanol ester and surfactant(s), is used to reduce cholesterol absorption from foods and beverages includes the step of commingling the food additive composition with foods and beverages, mixing until uniformly blended.

In a preferred method of commingling the indicated food additive with foods and beverages which contain cholesterol, the food additive is added such that the amount of stanols in the food additive is in the ratio of about 1:1 by weight to the cholesterol contained in the foods and beverages. Thus, for a food additive composition which comprises 25% stanols and a food which contains about 0.1% cholesterol (such as hamburger), the ratio of food additive to food product is about 1:250 by weight.

The food additive composition of the invention can be commingled with foods by a step selected from the group of infusion, injection, mixing, kneading, blending, immersion, spraying, surface application (for example, brushing and basting), cooking in oils which contain the food additive-invention, and combinations thereof. Preferred steps for commingling the food additive-invention with ground meat are kneading and mixing; for meat pieces such as steaks, chicken breasts, and chopped, diced or sliced meat, the preferred steps are injection, infusion, spraying, immersion, and surface applications such as basting and

marinating. Two preferred steps for commingling the food additive-invention with beverages are mixing and blending.

The compositions of the present invention will be used as food additives to foods such as meats, eggs and dairy products. Generally, when used as food additives, the compositions of the present invention will not contribute substantially to the taste of the food product. Accordingly, the compositions of the present invention can be used in food products without compromising the food products taste and flavor.

The compositions of the present invention may also be formulated into fine particles which may be sprinkled on to other food products, i.e., dairy products such as ice cream or candy.

The compositions of the invention may be administered to any animal. Foremost among such animals are mammals, e.g., humans, although the invention is not intended to be so limited. The dosage administered will be dependent upon the age, health, and weight of the recipient, kind of concurrent treatment, if any, frequency of treatment, and the nature of the effect desired.

The compositions of the present invention may be administered orally in any of the usual solid forms such as pills, tablets, capsules or powders, including sustained release preparations. The term unit dosage form as used in this specification and the claims refer to physically

discrete units to be administered in single or multiple dosage to animals, each unit containing a predetermined quantity of active material, i.e., phytostanol and/or phytostanol ester, in association with a surfactant(s) and a carrier. The quantity of active material is that calculated to produce the desired therapeutic effect upon administration of one or more of such units. Of course, it is understood that the exact treatment level will depend upon the case history of the animal, e.g., human being, that is treated. The precise treatment level can be determined by one of ordinary skill in the art without undue experimentation.

The require dosage of the phytostanol and/or phytostanol ester will vary with the severity of the condition and the duration of the treatment. Unit dosages can range from about 0.01 mg/kg to about 500 mg/kg (the unit designated "mg/kg" as used herein refers to mg of phytostanol and/or phytostanol ester per kilogram of body weight), preferably from about 0.1 mg/kg to about 125 mg/kg with up to six doses daily, preferably four dosages daily. Most preferably, the doses are administered at meal times. The dosages may be administered orally in any suitable unit dosage form such as pills, tablets, and capsules. Preferred are capsules made from gelatin.

As used herein, the term "carrier" denotes a solid or liquid filler, diluent, or encapsulating substance. Some examples of the substances that can act as carriers are sugars such as lactose, glucose, and sucrose; starches such as corn

starch and potato starch; cellulose and its derivatives, such as sodium carboxymethylcellulose, ethylcellulose, cellulose acetate; powdered tragacanth; malt; gelatin; talc; stearic acid; magnesium stearate; calcium sulfate; vegetable oils, such as peanut oil, cottonseed oil, sesame oil, olive oil, corn oil and of the broma; polyols such as propylene glycol, glcerin, sorbitol, mannitol, and polyethylene glycol; agar, alginic acid; pyrogen-free water; isotonic saline; ethyl alcohol and phosphate buffer solutions, as well as other non-toxic compatible substances used in preparation of formulations. Wetting agents and lubricants such as sodium lauryl sulfate, as well as coloring agents, flavoring agents, and preservatives can also be present. Dye stuffs or pigments may be added to the tablets, for example, for identification or in order to characterize combinations of active doses

Other preparations that can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer such as glycerol or sorbitol. The push-fit capsules can contain the active compounds in the form of granules, which may be mixed with fillers such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds are preferably dissolved or suspended in suitable liquids, such as fatty oils, or liquid paraffin. In addition, stabilizers may be added.

Powders are prepared by comminuting the compositions of the

present invention to a suitable fine size and mixing with a similarly comminuted diluent pharmaceutical carrier such as an edible carbohydrate material as for example, starch. Sweetening, flavoring, preservative, dispersing and coloring agents can also be present.

Capsules are made by preparing a powder mixture as described above and filling formed gelatin sheaths. A lubricant such as talc, magnesium stearate and calcium stearate can be added to the powder mixture as an adjuvant before the filling operation; a glidant such as colloidal silica may be added to improve flow properties; a disintegrating or solubilizing agent may be added improve the availability of the medicament when the capsule is ingested.

Tablets are made by preparing a powder mixture, granulating or slugging, adding a lubricant and disintegrant and pressing into tablets. A powder mixture is prepared by mixing the compositions of the present invention, suitable comminuted, with a diluent or base such as starch, sucrose, kaolin, dicalcium phosphate and the like. The powder mixture can be granulated by wetting with a binder such as syrup, starch paste, acacia mucilage or solutions of cellulosic or polymeric materials and forcing through a screen. As an alternative to granulating, the powder mixture can be run through the tablet machine and the resulting imperfectly formed slugs broken into granules. The granules can be lubricated to prevent sticking to the tablet forming dies by means of the addition of stearic acid, a stearate salt, talc or mineral oil. The lubricated

mixture is then compressed into tablets. The medicaments can also be combined with free flowing inert carriers and compressed into tablets directly without going through the granulating or slugging steps. A protective coating consisting of a sealing coat of shellac, a coating of sugar or polymeric material and a polish coating of wax can be provided. Dye stuffs or pigments may be added to the tablets, for example, for identification or in order to characterize combinations of active doses. In tablet form the carrier comprises from about 0.1% to 99% by weight of the total composition.

This invention will be better understood from the Examples which follow. However, one skilled in the art will readily appreciate that the specific methods and results discussed are merely illustrative of the invention and no limitation of the invention is implied.

EXAMPLE 1

Soy sitostanol (AC Humko, NF00114) (1g) was physically mixed with an equal weight of sodium dioctylsulfosuccinate (Aldrich Chemicals). The blend was melted at 170°C to form a clear solution. The clear solution was rapidly cooled by pouring it into liquid nitrogen. A crystalline transparent salt was obtained. The salt (2g) was added to water (8g) to give a milky-white emulsion with no visible particles. The solubility of the crystalline was found to be 1200 micrograms per ml using the dissolution assay described below. (Maximum solubility of cryground beta-sitostanol in

the dissolution assay was found to be 300 micrograms per ml.)

Dissolution Assay

A stock dissolution solution (5X) was prepared comprising taurocholic acid, Na^{2+} (500 mM, Sigma, cat# T4009), 2-monoolein (10 mM, Nu-Check Prep, cat# M-239), oleic acid, free (10 mM, Sigma cat# O1008) in cholorform:MeOH (1:1). A buffer solution was prepared comprising 0.01M sodium phosphate buffer with 3 mM sodium azide, pH 7.4.

In order to prepare the dissolution solution for use in the dissolution assay 1 ml of the 5X stock dissolution solution in a 20 ml vial was dried down under an N_2 stream at 50°C. The dried down dissolution solution was then redried twice from 2 ml ethyl ether with vortexing of the vial following each addition. Excess ether was then driven off by drying the vial for 1 hour at 100°C in an oven.

The vial with the dried down dissolution solution was rehydrated with 5 ml of the sodium phosphate buffer. Nitrogen was bubbled through the solution for 5 minutes to ensure complete removal of ether. The final concentrations of the dissolution constituents were taurocholic acid (100 mM), 2-monoolein (2 mM), oleic acid (2 mM) in sodium phosphate (0.01 M, pH 7.4) with 3 mM NaN_3.

In order to determine the solubility of the blend of the present Example, a sample of the blend having an equivalent of 100 mg sitostanol was added to the vial with the 5 ml of

the rehydrated dissolution solution. The contents of the vial were gently swirled and then placed in rotating incubator. A 100 mg cholesterol sample was prepared for assaying in the same manner as the blend of the present Example, as well as a 100 mg sample of cryoground stanol. At 4 hours a 1.0 ml aliquot was removed from each vial. The aliquots were immediately filtered through a 0.2 μm syringe filter.

Using a Cholesterol CII Enzymatic Kit (Wako Pure Chemical Industries, Ltd.) the amount of stanol dissolved in the filtered dissolution solution was determined according to the following protocol. Note that the concentration of stanol is given as the amount of unesterified stanol.

1. A 2X color reagent was prepared by adding only one-half the recommended buffer solution to the supplied lyophilized color reagent.

2. A 0.2 mg/ml sitostanol standard was prepared by aliquoting sitostanol from a 1:1 $CHCl_3$/MEOH stock and adding triton-X 100 for a final rehydrated concentration of 2.0% Triton, 0.2 mg/ml sitostanol. This solution was vortexed and dried down under a N_2 stream at 50°C, followed by redrying twice from ethyl ether. The sample was rehydrated in of 0.01 M sodium phosphate buffer with 3 mM NaN_3, pH 7.4. The mixture was then vortexed.

4. A 96 well plate was setup. The first two columns were used for the standard. Each sample was run in quadruplicate.

5. Samples for a standard curve were formed by adding water to the well first followed by the standard according to the proportions outlined in the table below:

Water (ml)	Sitostanol Standard (ml)	Sitostanol (mg)
100	0	0
97.5	2.5	0.5
95	5	1
90	10	2
80	20	4
70	30	6
60	40	8
50	50	10

6. Samples (5 microliter) were added to wells containing water so that the final volume was 100 ml.

7. The 2X color reagent was added to each of the wells using a multi-pipettor. Care should be taken to avoid contact with the solutions by touching the pipet tips at the top edges of the well wall.

8. The reagents were then mixed by shaking on a plate shaker (cover with plate sealing plastic). The plates were incubated at 37°C for 15 min.

9. The plate was removed from the incubator and an ELISA reader at 500 nm (within 1 hour of plates removal from the incubator) was used to read the plate and thereby determine the solubility of the sample in the dissolution solution.

EXAMPLE 2

The formulation (20g) of Example 1 was dispersed in water (100 mL) in a high speed blender prior to mixing with hamster chow containing 10% by weight corn oil (0.24% cholesterol). The dispersion was added to the hamster chow to give 2% by weight stanol equivalent. Hamsters were fed diet for 7 days and fecal cholesterol levels were determined on the last 48 hours pooled samples. Fecal cholesterol ratio ("FC Ratio") for each sample, formulated stanol and unformulated stanol (control), is determined by dividing their cholesterol measurement by the cholesterol measurement for hamsters fed hamster chow with 10% by weight corn oil and 0.24% cholesterol (containing chow only). Comparison of FC Ratios relative to the control, 2% by weight of unformulated stanol equivalent, showed that the hamsters fed the chow with the formulation from Example 1 absorbed less cholesterol, i.e., larger FC ratio. The results are shown in Table 1.

Formulation	FC Ratio
Control	3.1
Example 1	3.8

Table 1

Additional studies were conducted using dispersions of the formulation prepared in Example 1 having 1% by weight stanol equivalent in hamster chow as described above. Results indicated that chow with 1% by weight stanol equivalent had enhanced fecal cholesterol ratios relative to the control. Note that some variability between FC Ratios will be observed due to the use of different hamsters in studies. The results are shown in Table 2.

Formulation	FC Ratio
Control	3.1
Example 1	4.4

Table 2

EXAMPLE 3

Evaluation of the efficacy of the formulations of the present invention to inhibit the absorption of cholesterol in the intestine was conducted using male beagle dogs. The dogs had an approximate weight of 8 to 12 kg at the start of the study and were approximately 11 to 13 months old at the time of purchase. The dogs were housed in individual,

stainless steel cages. The cages were modified for the separation of urine and feces and the collection of feces during the fecal collection periods.

During the acclimation and washout periods the dogs were fed certified canine diet #5007 (PMI Feeds, Inc.). During the testing period, days 1-4, the dogs were fed certified canine diet #5007, supplemented with 0.25% cholesterol made by Ed Uhlman at Research Diets, Inc. New Brunswick, NJ. All diets were offered for approximately 2 to 4 hours daily at approximately the same time each day. Water was provided ad libitum. The animal room's environment was maintained at a temperature of 18° to 29°C, a relative humidity of 50% ± 20%, and a 12-hour light/12-hour dark cycle.

The following formulations were tested: a control sample of stanol in a push-fit gelatin capsule and a 50 weight % cremophor/50 weight % soy stanol blend, which was prepared by co-melting the cremophor and stanol together at 180°C and rapidly quenching the melt in liquid nitrogen to afford a transparent crystalline salt. The salt was ground to a fine powder using a cryo-mill and placed in a push-fit gelatin capsule for administration. A 9% AOT/9% PEG/72% soy sitostanol blend formulation by solution blending in chloroform. The chloroform was then evaporated to give an amorphous material, which was cryo-milled into a fine powder. The powder was placed in a push-fit gelatin capsule for administration. Animals were dosed at 63 mg/kg stanol equivalence.

Each animal received the cholesterol-supplemented diet and the formulations of the present invention in gelatin capsule form daily for 4 days. The gelatine capsules and controls were offered at approximately the same time each day, before the animals were fed in on each day.

Pre-test fecal samples were collected for 72 hours before test formulation administration and on test days 3, 4 and 5. Samples were collected before feed in (and before administration of the samples and control) each day, transferred to plastic containers, and pooled at 3-day intervals. The fecal cholesterol level in each of the collected samples was then determined using liquid chromatography mass-spectrometry ("LC-MS").

Formulation (Ref.)	FC Ratio
Control (Stanol)	0.95
50% Cremophor/50% stanol (A)	1.43
9% AOT/9% PEG/72% stanol (B)	1.95

Table 3

EXAMPLE 4

Ingredient	Amount (mg)
Stanol	600
1-Octadecanol	31.58
Ac-Di-Sol	91.58
Crodesta F160	126.32
Stearic Acid	1.58

Stanol, 1-octadecanol (Aldrich, Milwaukee, WI), and half of the Ac-Di-Sol (croscarmellose sodium NF Type A, FMS Corp., Newark, Delaware) were mixed in a V-blender (Model # Twin Shell Dry Blender 4QT, Patterson-Kelly Co., East Stroudsbourgh, PA) for 5 minutes into a powder mixture. The resulting mixture was compacted in a TF-Miniroller compactor (Model No. TF-Mini, Vector Corp., Marion, Iowa; 180 kg/cm^2 of pressure, feed speed of 10 RPM and roller speed of 7 RPM) resulting in sintering and melting of the mixture. The compacted material was then passed through a Comil (Model No. 197.5, Quadro Engineering Inc., Waterloo, Canada). The powder was then mixed with the reminder of the Ac-Di-Sol and Crodesta F-160 (sucrose stearate, Croda, Inc., Mill Hall, PA), followed by blending with the V-blender for 5 minutes. The stearic acid (triple pressed, Mallinckrodt Baker, Inc., Phillipsbury, NJ), which was first passed through a 40 mesh sieve, was added to the blend in the V-blender and mixed for 3 minutes. The resulting mixture is then tableted by compression in a standard tablet press.

The fecal cholesterol ratio for this formulation was compared to Benecol as a control. The results are shown in the following table:

Formulation	FC Ratio
Control (Benecol)	1.15
Example 4	1.08

Table 4

EXAMPLE 5

Push-fit capsules comprising a unit dosage form of a stanol were prepared as described in Example 4, having the following composition,

Ingredient	Amount (mg)
Stanol	600
Crodesta F160	120
Ac-Di-Sol	90
Avicel PH-102	25
Sodium Palmitate	50
Stearic Acid	6

Avicel PH-102 is microcrystalline cellulose and is available from FMC Corp., Newark, Delaware.

The fecal cholesterol ratio for this formulation was compared to Benecol as a control. Benecol is a spread that incorporates plant stanol esters, as a method to promote healthy cholesterol levels and is available from McNeil Consumer Healthcare, Ft. Washington PA. The results are shown in the following table:

Formulation	FC Ratio
Control (Benecol)	1.15
Example 5	1.14

Table 5

EXAMPLE 6

Push-fit capsules comprising a unit dosage form of a stanol were prepared as described in Example 4, having the following composition,

Ingredient	Amount (mg)
Stanol	600
1-Octadecanol	30
Hydroxylated Lecithin	60
Sodium Palmitate	60
Ac-Di-Sol	85
Avicel PH-102	25
Crodesta F160	10

The fecal cholesterol ratio for this formulation was compared to Benecol as a control. The results are shown in the following table:

Formulation	FC Ratio
Control (Benecol)	1.15
Example 6	1.16

Table 6

EXAMPLE 7

Push-fit capsules comprising a unit dosage form of a stanol were prepared as described in Example 4, having the following composition,

Ingredient	Amount (mg)
Stanol	380
1-Octadecanol	20
Hydroxylated Lecithin	50
Ac-Di-Sol	38
Crodesta F160	42
Avicel PH-102	14
Stearic Acid	3

The fecal cholesterol ratio for this formulation was compared to Benecol as a control. The results are shown in the following table:

Formulation	FC Ratio
Control (Benecol)	1.10
Example 7	1.30

Table 7

EXAMPLE 8

Deoiled Hydroxylated lecithin (10g, Central Soya) was dispersed in water (30g) using a high speed blender. Hammer-milled stanol (20g) was added to the dispersion and the mixture was once again blended under high shear. The

resulting mixture was dried at 70°C at reduced pressure (6 inches, Hg) overnight in a vacuum oven. The resulting mixture was hammer-milled to obtain a fine powder. The solubility of the powder was found to be 750 mg per mL using the dissolution assay described in Example 1.

A push-fit gelatin capsule was prepared using the powder having a dosage of 63 mg stanol per kg of dog weight. The efficacy of the prepared gelatin capsule containing the powder was compared to a benecol at the same stanol dosage level. The benecol was delivered to the dog in liquid form using a syringe. The FC ratio for the dog dosed with powder prepared in this Example was determined and compared to the FC ratio for a control dog dosed with benecol. The results are shown in Table 8.

Formulation	FC Ratio
Control (Benecol)	1.17
Example 8 (stanol/hydroxylated lecithin)	1.57

Table 8

EXAMPLE 9

A formulation containing lecithin in place of hydroxylated lecithin was formulated as in Example 8. The FC rations determined for the formulation compared to Benecol from a dog study administered in the same manner as Example 8 are shown below:

Formulation	FC Ratio
Control (Benecol)	1.29
Example 9 (stanol/lecithin)	1.3

Table 9

EXAMPLE 10

Compressed tablets comprising a unit dosage form of a stanol were prepared as described in Example 4, having the following composition,

Ingredient	Amount (mg)
Stanol	600
Crodesta F160	88.42

Ac-Di-Sol	60
Avicel PH-102	49.26
Stearic Acid	5.68
Tween 60	31.58
PEG 3350 Powder	30.32

Table 10

The fecal cholesterol ratio for this formulation was compared to Benecol as a control. The results are shown in the following table:

Formulation	FC Ratio
Control (Benecol)	1.06
Example 10	1.14

Table 11

EXAMPLE 11

33.47 grams of the ocadecanoic acid ester of stanol was dissolved in 250 ml of chloroform. 1.05 grams of octadecanol was dissolved added to chloroform solution with heating at 51 degrees C to facilitate dissolution. The chloroform was removed by evaporation under nitrogen yielding a solid mixture containing 3.04% octadecanol. The mixture was cryo-ground into hamster chow and fed to hamsters at 1 percent stanol equivalent in their diet. The

hamsters who consumed the chow with stanol ester were found to have absorbed less cholesterol than a control group of hamsters that eat hamster chow without stanol ester.

Example 12

Hydroxylated lecithin powder (Precept 8120, Central Soya) was combined with stanol powder (AC Humko NF00114) to give a mixture containing 70% w/w stanol and 30% w/w hydroxylated lecithin. The mixture was extruded using a Prism twin screw which allows for temperature control over four zones and the die end. For this example zone temperatures 1 through 4 were set to 65°C. The die temperature was set to 90°C and the screw speed set to 200 rpm. Approximately 100 grams of this material was extruded in long spaghetti like rods which were too soft and brittle to be processed through a chopper. The rods were hammer milled into a free flowing powder under liquid nitrogen. Hard gelatin capsules were filled with the powdered lecithin/stanol product and tested for cholesterol absorption inhibition in the standard dog model described above in Example 3. Dose-response results are shown in Table 12, indicating performance statistically better than control at 30, 120 and 360 mg/Kg.

Formulation	Cholesterol Excreted (μg/g feces/day)
Control	656.03
Benecol (60 mg/kg)	867.53
Example 12 (30 mg/kg)	814.88
Example 12 (60 mg/kg)	758.03
Example 12 (120 mg/kg)	1083.19
Example 12 (360 mg/kg)	1114.65

Table 12

Example 13

Sodium stearate (Witco) was added to a 70:30 mixture of stanol:hydroxylated lecithin powder prepared as in Example 12 to give 16.7% w/w stearate. The powders were fed into a Prism twin screw extruder using a vibrating feeder. Temperatures of zones 1 to 4 were set at 50, 57, 68 and 91°C respectively (the actual temperatures measured in zones 2-4 were 57, 69 and 99°C, respectively). The die temperature was set to 110°C (actual temperature 102°C). The screw speed was set to 100 RPM. As in Example 12, spaghetti-like rods were extruded and hammer milled to yield a free-flowing powder. The powder was incorporated into gelatin capsules and tested for cholesterol absorption in the dog model discussed in Example 3.

Example 14

A mixture of stanol 62% (w/w) (AC Humko), hydroxylated lecithin 30% (w/w) (Precept 8120, Central Soya) and Ac-Di-Sol 8% (w/w)(croscarmellose sodium NF Type A, FMS Corp., Newark, Delaware) was compressed using roller compaction and then co-milled. The resultant powder had sufficient bulk density to enable filling a gel capsule with at least 400 mg of stanol. The material was tested for cholesterol absorption in the dog model discussed in Example 3.

Formulation	Cholesterol Excreted (μg/g feces/day)
Control	397
Benecol (60 mg/kg)	477
Benecol Soft Gel (60 mg/kg)	534
Example 14 (60 mg/kg)	554
Example 14 (120 mg/kg)	550
Example 14 (240 mg/kg)	653

Table 13

Example 15

The following tablet formulations were prepared.

Formulation A

Ingredient	Conc. % (w/w)	mg/tablet
Spray chilled stanol/octadecanol	65.80	526.4
Sucrose, NF (SugarTab)	16.66	133.3
Microcrystalline Cellulose, NF (Avicel PH-102)	11.00	88.0
Croscarmellose Sodium, NF (Ac-Di-Sol)	6.00	2.7
Sodium Lauryl Sulfate, NF	0.34	2.7
Stearic Acid, NF	0.20	1.6
Totals	100.00	800.0

Formulation B

Ingredient	Conc. % (w/w)	mg/tablet
Spray chilled stanol/octadecanol /Tween-60[1]	69.5	556.0
Sucrose, NF (SugarTab)	13.3	106.4
Microcrystalline Cellulose, NF (Avicel PH-102)	11.0	88.0
Croscarmellose Sodium, NF (Ac-Di-Sol)	6.0	48.0
Stearic Acid, NF	0.2	1.6
Totals	100.0	800.0

[1]Equivalent to 27.8 mg for each of the octadecanol and Tween 60 per tablet.

Formulation C

Ingredient	Conc. % (w/w)	mg/tablet
Spray chilled stanol/octadecanol	74.05	631.6
Sucrose Monostearate, FCC (Crodesta F-160)	14.81	126.3
Croscarmellose Sodium, NF (Ac-Di-Sol)	10.74	91.6
D&C Yellow #10 Lake	0.21	1.8
Stearic Acid, NF	0.19	1.6
Totals	100.00	852.9

Formulation D

Ingredient	Conc. % (w/w)	mg/tablet
Spray chilled stanol/octadecanol	36.72	315.79
Sucrose, NF (SugarTab)	50.09	430.79
Sucrose Monostearate, FCC (Crodesta F-160)	7.34	63.16
Croscarmellose Sodium, NF (Ac-Di-Sol)	5.32	45.79
D&C Yellow #10 Lake	0.24	2.10
Stearic Acid, NF	0.28	2.37
Totals	100.00	860.00

Other objects, advantages, features, modifications of this invention will be apparent to those of ordinary skill in this art. This invention is not limited except as set forth in the following claims.

What is claimed is:

1. A composition comprising a mixture of a phytostanol and a surfactant selected from the group consisting of sodium docusate, ammoniated glycyrrhizin, polyoxyethylene castor oil, polyethylene glycol, diacetyl lactic acid esters of mono- and diglycerides, diacetyl tartaric acid esters of mono- and diglycerides, monosodium phosphate derivatives of mono- and diglycerides, ethoxylated mono- and diglycerides, quillaja saponin, ethylene oxide propylene oxide block copolymers, vitamin E TPGS, hydroxylated lecithin and mixtures thereof.

2. The composition of claim 1, further comprising a surfactant selected from the group consisting of sodium salts of fatty acids, fatty alcohols, polyethylene glycol (8) stearate, polyethylene glycol (40) stearate, sucrose fatty acid esters, tween and mixtures thereof.

3. The composition of claim 1, wherein the phytostanol is selected from the group consisting of sitostanol, campestanol, 22,23 dihydrobrassicastanol, clionastanol and mixtures thereof.

4. The composition of claim 3, wherein the sitostanol is alpha-sitostanol or beta-sitostanol.

5. The composition of claim 2, wherein said fatty alcohol is selected from the group consisting of 1-decanol, 1-

dodecanol, 1-tetradecanol, 1-hexadecanol, 1-octadecanol, 9-octadecen-1-ol, 1-eicosanol, 1-docosanol, 1-hexacosanol, 1-octacosanol, 1-triacontanol and mixtures thereof.

6. The composition of claim 5, wherein said fatty alcohol is 1-octadecanol.

7. The composition of claim 2, wherein said sucrose fatty acid ester is selected from the group consisting of sucrose stearate, sucrose distearate, sucrose palmitate and mixtures thereof.

8. The composition of claim 2, wherein said tween is selected from the group consisting of polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, and polysorbate 85.

9. The composition of claim 1, comprising from about 10 to about 99.99 weight percent of said phytostanol and from about 0.01 to about 90 weight percent of said surfactant.

10. The composition of claim 9, comprising from about 40 to about 95 weight percent of said phytostanol and from about 5 to 60 weight percent of said surfactant.

11. The composition of claim 10, comprising about 95 weight percent of said phytostanol and about 5 weight percent of said surfactant.

12. A method of reducing cholesterol absorption in humans which comprises orally administering an effective amount of a mixture of a phytostanol and a surfactant selected from the group consisting of sodium docusate, ammoniated glycyrrhizin, polyoxyethylene castor oil, polyethylene glycol, diacetyl lactic acid esters of mono- and diglycerides, diacetyl tartaric acid esters of mono- and diglycerides, monosodium phosphate derivatives of mono- and diglycerides, ethoxylated mono- and diglycerides, quillaja saponin, ethylene oxide propylene oxide block copolymers, vitamin E TPGS, hydroxylated lecithin and mixtures thereof.

13. The method of claim 12, further comprising a surfactant selected from the group consisting of sodium salts of fatty acids, fatty alcohols, polyethylene glycol (8) stearate, polyethylene glycol (40) stearate, sucrose fatty acid esters, tween and mixtures thereof.

14. The method of claim 12, wherein the phytostanol is selected from the group consisting of sitostanol, campestanol, 22,23 dihydrobrassicastanol, clionastanol and mixtures thereof.

15. The method of claim 14, wherein the sitostanol is alpha-sitostanol or beta-sitostanol.

16. The method of claim 13, wherein said fatty alcohol is selected from the group consisting of 1-decanol, 1-

dodecanol, 1-tetradecanol, 1-hexadecanol, 1-octadecanol, 9-octadecen-1-ol, 1-eicosanol, 1-docosanol, 1-hexacosanol, 1-octacosanol, 1-triacontanol and mixtures thereof.

17. The method of claim 16, wherein said fatty alcohol is 1-octadecanol.

18. The method of claim 13, wherein said sucrose fatty acid ester is selected from the group consisting of sucrose stearate, sucrose distearate, sucrose palmitate and mixtures thereof.

19. The method of claim 13, wherein said tween is selected from the group consisting of polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, and polysorbate 85.

20. The method of claim 12, comprising from about 10 to about 99.99 weight percent of said phytostanol and from about 0.01 to about 90 weight percent of said surfactant.

21. The method of claim 20, comprising from about 40 to about 95 weight percent of said phytostanol and from about 5 to 60 weight percent of said surfactant.

22. The method of claim 21, comprising about 95 weight percent of said phytostanol and about 5 weight percent of said surfactant.

23. The method of claim 12, wherein said mixture is orally administered in the form of chewable, effervescent, swallowable and coated tablets, capsules, soft gelatine capsules, syrup, fruit beverages, granule sachets, fruit gelatines, mini-sweets or sweets.

24. A method for reducing serum cholesterol levels comprising administering a mixture of a phytostanol and a surfactant selected from the group consisting of sodium docusate, ammoniated glycyrrhizin, polyoxyethylene castor oil, polyethylene glycol, diacetyl lactic acid esters of mono- and diglycerides, diacetyl tartaric acid esters of mono- and diglycerides, monosodium phosphate derivatives of mono- and diglycerides, ethoxylated mono- and diglycerides, quillaja saponin, ethylene oxide propylene oxide block copolymers, vitamin E TPGS, hydroxylated lecithin and mixtures thereof.

25. The method of claim 24, further comprising a surfactant selected from the group consisting of sodium salts of fatty acids, fatty alcohols, polyethylene glycol (8) stearate, polyethylene glycol (40) stearate, sucrose fatty acid esters, tween and mixtures thereof.

26. The method of claims 24 or 25, wherein the mixture is administered orally.

27. The method of claim 24, wherein the phytostanol is selected from the group consisting of sitostanol, campestanol, 22,23 dihydrobrassicastanol, clionastanol and mixtures thereof.

28. The method of claim 27, wherein the sitostanol is alpha-sitostanol or beta-sitostanol.

29. The method of claim 25, wherein said fatty alcohol is selected from the group consisting of 1-decanol, 1-dodecanol, 1-tetradecanol, 1-hexadecanol, 1-octadecanol, 9-octadecen-1-ol, 1-eicosanol, 1-docosanol, 1-hexacosanol, 1-octacosanol, 1-triacontanol and mixtures thereof.

30. The method of claim 29, wherein said fatty alcohol is 1-octadecanol.

31. The method of claim 25, wherein said sucrose fatty acid ester is selected from the group consisting of sucrose stearate, sucrose distearate, sucrose palmitate and mixtures thereof.

32. The method of claim 25, wherein said tween is selected from the group consisting of polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, and polysorbate 85.

33. The method of claim 24, comprising from about 10 to about 99.99 weight percent of said phytostanol and from

about 0.01 to about 90 weight percent of said surfactant.

34. The method of claim 33, comprising from about 40 to about 95 weight percent of said phytostanol and from about 5 to 60 weight percent of said surfactant.

35. The method of claim 34, comprising about 95 weight percent of said phytostanol and about 5 weight percent of said surfactant.

36. A method for preparing a composition for the reduction of cholesterol absorption, comprising the step of mixing a phytostanol and a surfactant selected from the group consisting of sodium docusate, ammoniated glycyrrhizin, polyoxyethylene castor oil, polyethylene glycol, diacetyl lactic acid esters of mono- and diglycerides, diacetyl tartaric acid esters of mono- and diglycerides, monosodium phosphate derivatives of mono- and diglycerides, ethoxylated mono- and diglycerides, quillaja saponin, ethylene oxide propylene oxide block copolymers, vitamin E TPGS, hydroxylated lecithin and mixtures thereof.

37. The method of claim 36, wherein the mixing is conducted under elevated pressure.

38. The method of claim 37, wherein the mixing is conducted by roller compaction.

39. The method of claim 37, wherein the mixing is conducted in an extruder.

40. The method of claim 36, further comprising a surfactant selected from the group consisting of sodium salts of fatty acids, fatty alcohols, polyethylene glycol (8) stearate, polyethylene glycol (40) stearate, sucrose fatty acid esters, tween and mixtures thereof.

41. The method of claim 40, further comprising the step of heating said mixture of said phytostanol and said surfactants to a temperature that results in the formation of a melt.

42. The method of claim 41, further comprising the step of rapidly cooling said melt.

43. The method of claim 42, wherein liquid nitrogen is used for cooling.

44. A food product comprising a mixture of a phytostanol and a surfactant selected from the group consisting of sodium docusate, ammoniated glycyrrhizin, polyoxyethylene castor oil, polyethylene glycol, diacetyl lactic acid esters of mono- and diglycerides, diacetyl tartaric acid esters of mono- and diglycerides, monosodium phosphate derivatives of mono- and diglycerides, ethoxylated mono- and diglycerides, quillaja saponin, ethylene oxide propylene oxide block

copolymers, vitamin E TPGS, hydroxylated lecithin and mixtures thereof.

45. The food product of claim 44, further comprising a surfactant selected from the group consisting of sodium salts of fatty acids, fatty alcohols, polyethylene glycol (8) stearate, polyethylene glycol (40) stearate, sucrose fatty acid esters, tween and mixtures thereof.

46. The food product of claim 44, wherein the phytostanol is selected from the group consisting of sitostanol, campestanol, 22,23 dihydrobrassicastanol, clionastanol and mixtures thereof.

47. The food product of claim 46, wherein the sitostanol is alpha-sitostanol or beta-sitostanol.

48. The food product of claim 45, wherein said fatty alcohol is selected from the group consisting of 1-decanol, 1-dodecanol, 1-tetradecanol, 1-hexadecanol, 1-octadecanol, 9-octadecen-1-ol, 1-eicosanol, 1-docosanol, 1-hexacosanol, 1-octacosanol, 1-triacontanol and mixtures thereof.

49. The food product of claim 48, wherein said fatty alcohol is 1-octadecanol.

50. The food product of claim 45, wherein said sucrose fatty acid ester is selected from the group consisting

of sucrose stearate, sucrose distearate, sucrose palmitate and mixtures thereof.

51. The food product of claim 45, wherein said tween is selected from the group consisting of polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, and polysorbate 85.

52. The food product of claim 44, comprising from about 10 to about 99.99 weight percent of said phytostanol and from about 0.01 to about 90 weight percent of said surfactant.

53. The food product of claim 52, comprising from about 40 to about 95 weight percent of said phytostanol and from about 5 to 60 weight percent of said surfactant.

54. The food product of claim 53, comprising about 95 weight percent of said phytostanol and about 5 weight percent of said surfactant.

55. A composition comprising a mixture of a phytostanol ester and a surfactant selected from the group consisting of sodium docusate, ammoniated glycyrrhizin, polyoxyethylene castor oil, polyethylene glycol, diacetyl lactic acid esters of mono- and diglycerides, diacetyl tartaric acid esters of mono- and diglycerides, monosodium phosphate derivatives of mono- and diglycerides, ethoxylated mono- and diglycerides, quillaja saponin, ethylene oxide propylene oxide block

copolymers, vitamin E TPGS, hydroxylated lecithin and mixtures thereof.

56. The composition of claim 55, further comprising a surfactant selected from the group consisting of sodium salts of fatty acids, fatty alcohols, polyethylene glycol (8) stearate, polyethylene glycol (40) stearate, sucrose fatty acid esters, tween and mixtures thereof.

57. The composition of claim 55, wherein said fatty alcohol is selected from the group consisting of 1-decanol, 1-dodecanol, 1-tetradecanol, 1-hexadecanol, 1-octadecanol, 9-octadecen-1-ol, 1-eicosanol, 1-docosanol, 1-hexacosanol, 1-octacosanol, 1-triacontanol and mixtures thereof.

58. The composition of claim 57, wherein said fatty alcohol is 1-octadecanol.

59. The composition of claim 56, wherein said sucrose fatty acid ester is selected from the group consisting of sucrose stearate, sucrose distearate, sucrose palmitate and mixtures thereof.

60. The composition of claim 56, wherein said tween is selected from the group consisting of polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, and polysorbate 85.

61. The composition of claim 55, comprising from about 10 to about 99.99 weight percent of said phytostanol ester and from about 0.01 to about 90 weight percent of said surfactant.

62. The composition of claim 61, comprising from about 40 to about 95 weight percent of said phytostanol ester and from about 5 to 60 weight percent of said surfactant.

63. The composition of claim 62, comprising about 95 weight percent of said phytostanol ester and about 5 weight percent of said surfactant.

INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/US 00/30417

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A23L1/30 A23L1/035

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A23L A23D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 897 671 A (UNILEVER PLC ;UNILEVER NV (NL)) 24 February 1999 (1999-02-24) cited in the application	1,3,4, 8-10,24, 26-28, 32-34, 36,41, 42,44, 46,47, 52,53, 55,61,62
	claims 1,4-7,10,15,16,25-27; examples 1,3,6 page 4, line 2-13,52 -page 5, line 1,9-19 page 7, line 4-12	
A		2,5-7, 11,25, 29-31, 35, 37-40, 45, 48-51,

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 March 2001	14. 03. 2001

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/30417

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
		54, 56-60,63

P,X	WO 00 47213 A (UNIV WASHINGTON) 17 August 2000 (2000-08-17)	1,3,4,9, 10,24, 26-28, 33,34, 36,37, 41,42, 44,46, 47,52,53
	claims 1,5,11-14; examples 1,6; tables 1,6 page 4, line 22 -page 5, line 9,15-27	
A	page 6, line 22 -page 7, line 15	2,5-8, 11,25, 29-32, 35, 38-40, 43,45, 48-51, 54-63

A	US 5 244 887 A (STRAUB CARL D) 14 September 1993 (1993-09-14) cited in the application claims 1,2 column 1, line 8-12 column 4, line 40-47 column 6, line 10-22 column 7, line 10-17	1-11, 24-63

A	EP 0 289 636 A (ASAHI DENKA KOGYO KK ;AJINOMOTO KK (JP)) 9 November 1988 (1988-11-09) claims 1,2,7; examples 1,2; table 1 page 2, line 8-11,50 -page 3, line 22,40-52	1-11, 24-63

P,A	WO 00 45648 A (FORBES MEDI TECH INC) 10 August 2000 (2000-08-10) claims 1,3,4; example 2 page 1, paragraph 1 page 7, paragraph 1 page 10, line 4 -page 11, line 1,2 page 12, paragraph 4 -page 13, paragraph 2	1-11, 24-63

P,A	EP 0 986 962 A (MCNEIL PPC INC) 22 March 2000 (2000-03-22) claims 1,4,5,7,9,10 page 2, line 34,35 page 3, line 1-5,19	1-11, 24-63

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/30417

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 12-23
because they relate to subject matter not required to be searched by this Authority, namely:
Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/30417

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